Axenic Culture and Induction of Callus and Sporophytes of the Appalachian Vittaria Gametophyte

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Asexually-reproducing gametophytes of the four tropical fern genera *Hymenophyllum*, *Trichomanes*, *Grammitis*, and *Vittaria* occur in the uplands of the southeastern United States (Farrar, 1967). The most common and abundant of these is the Appalachian *Vittaria* gametophyte, which Farrar (1978) has described in detail. Dr. A. J. Sharp of the University of Tennessee was the first to find and collect the Appalachian *Vittaria* gametophyte in the area of the Mountain Lake Biological Station in Virginia. Since that time, Dr. W. H. Wagner, Jr. of the University of Michigan collected it in the same area and catalogued it into a pteridophyte flora (Wagner, 1963). Other pteridologists have collected it in the area of the Highlands Biological Station in North Carolina (Wagner et al., 1970; Pittillo et al., 1975) and in Tennessee (Wofford & Evans, 1979). Other collections have located these gametophytes in several eastern states in and around the southern Appalachians (Farrar, 1978; Gastony, 1977).

Appalachian *Vittaria* gametophytes are long-lived and produce extensive colonies in deep, protected crevices of non-calcareous rock. Although antheridia and archegonia are produced on the gametophytes, reduced, juvenile sporophytes have been found only once in the field by Farrar (1978). They were recognizable as *Vittaria*, but lacked sufficient characters for identification to species. Since most taxonomic characters for determining fern species are based on sporophyte morphology, the origin and affinities of these gametophytes are uncertain. They were first assigned to the genus *Vittaria* by Wagner and Sharp (1963) based on finding similar morphological characters between the gametophytes of the Appalachian *Vittaria* and those of *Vittaria lineata* (L.) J. E. Smith from Florida. The chromosome counts reported by Gastony (1977) have provided further evidence of their relationship.

The experimental production of sporophytes might provide additional taxonomic characters for comparison with the *Vittaria* species. Farrar (1978) reported that Appalachian *Vittaria* gametophytes were difficult to maintain in axenic culture and ceased to grow on defined media. We report here successful axenic culture of the Appalachian *Vittaria* gametophyte and the spontaneous formation of callus and sporophytes.

MATERIALS AND METHODS

Gametophytes were collected from shaded, moist, sandstone conglomerate ledges along a cliff in Conkles Hollow State Park, Hocking County, Ohio. They were maintained under non-sterile conditions in culture dishes on washed perlite moist-

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ened with quarter-strength Knudson's medium (Steeves et al., 1955) without sugar. The dishes were maintained in a culture room at 25°C under a table where they received 25 foot-candles of diffuse white light consisting of a combination of fluorescent and incandescent lamps on an 18/6 hour light-dark photoperiod. Distilled water was added to the dishes as required. When the growing gametophytes half filled the dishes, fresh subcultures were initiated from small masses of gametophytes in freshly prepared culture dishes. At the moment, non-sterile cultures are being maintained in this manner.

Aseptic gametophyte cultures were obtained by placing a number of small gametophyte pieces into a 25 × 150 mm screw cap culture tube. About 25 ml of a 1% Alconox solution was added to wash and wet the gametophyte pieces, and the tube was agitated for two minutes. The Alconox solution was decanted, 35 ml of a 10% (V/V) Clorox solution was added, and the tube was agitated gently for one minute. In a UV-sterilized room, the gametophyte pieces were transferred from the Clorox solution to a 100 × 15 mm sterile, plastic Petri dish containing 30 ml of sterile, double-distilled water. Under continued sterile conditions, the gametophyte pieces were then transferred to 25 × 150 mm culture tubes which were plugged with cotton and a plastic cap (Kaput). Each tube contained 20 ml of Knudson's medium (Steeves et al., 1955) supplemented with 2% sucrose. One gametophyte piece was placed in each tube, with some in liquid media and others on media solidified with 0.8% agar. Some gametophyte pieces in liquid media were placed on filter paper supports above the medium. In an attempt to induce apogamy, gametophyte pieces were also cultured on media containing 0 to 4% sucrose in a series of cultures.

Aseptic gametophyte cultures can also be obtained from non-sterile stock cultures heavily contaminated with bacterial and fungal spores by forcing such spores to germinate before surface sterilization. Gametophyte pieces were floated on non-sterile, half strength Knudson's medium without sucrose and agar for one day. The gametophyte pieces were then surface-sterilized and placed into culture as described previously.

RESULTS AND DISCUSSION

Gametophytes in non-sterile cultures grew slowly in a concentric pattern. They produced gemmae, but did not produce either antheridia or archegonia.

Gametophytes in sterile culture showed signs of growth within two weeks of culture initiation, and slowly formed a near-spherical mass of ribbon-like prothallial lobes in each tube, whether in liquid or on agar-solidified media, over a period of several months. Based on visual inspection, it appeared that the gametophytes developed more rapidly on media containing 2% sucrose. Gametophytes on media containing sucrose concentrations above and below 2% developed at a reduced growth rate and appeared to be thinner and smaller than those exposed to 2% sucrose. Over a period of seven months, no gemmae or sex organs were observed in any cultures. Although the prothalli appeared normal in overall size and shape on media containing 2% sucrose, they produced an abundance of short, knob-like rhizoids (*Fig. 1*) in addition to the more normal, elongated rhizoids.

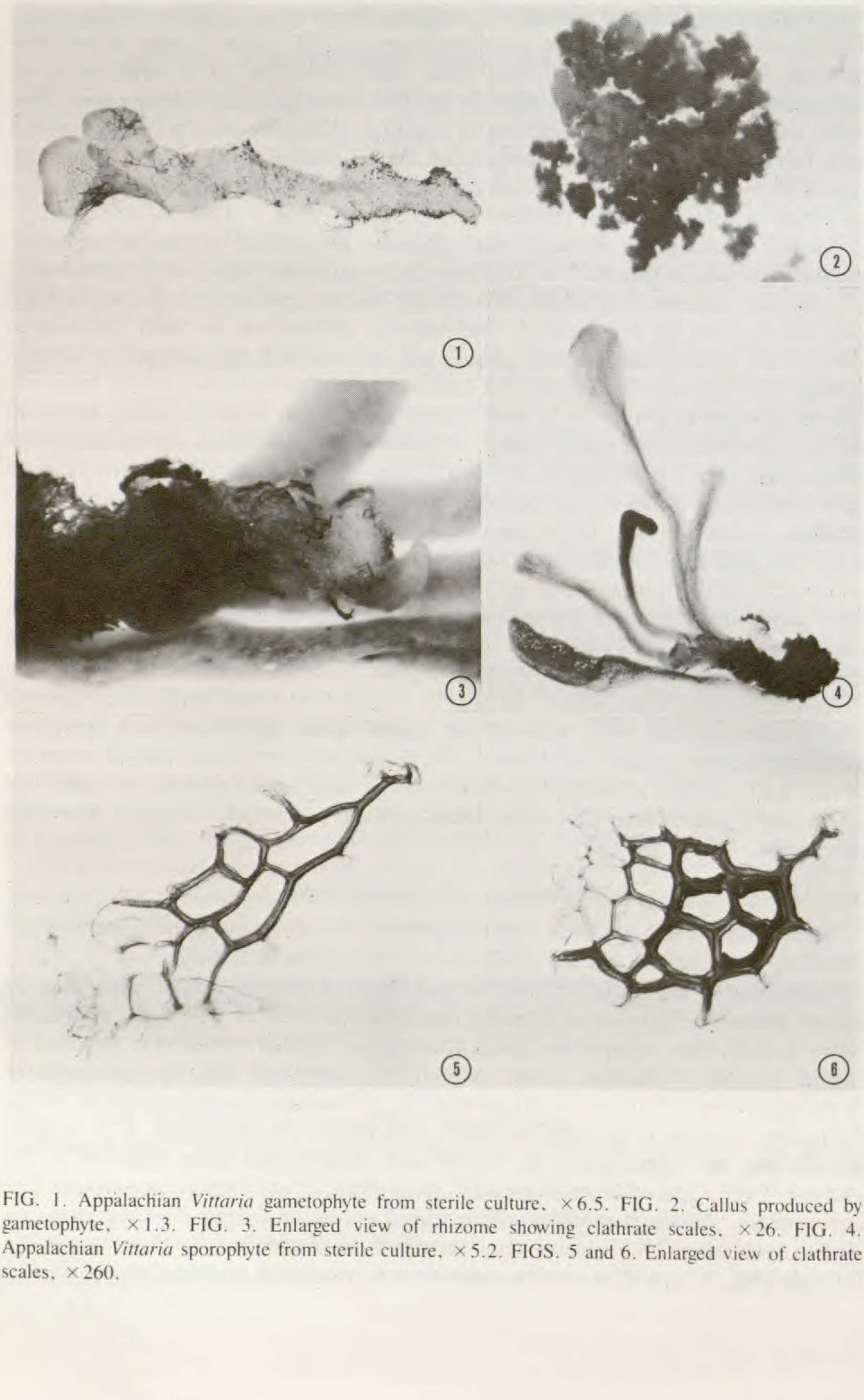

FIG. 1. Appalachian *Vittaria* gametophyte from sterile culture. ×6.5. FIG. 2. Callus produced by gametophyte. ×1.3. FIG. 3. Enlarged view of rhizome showing clathrate scales. ×26. FIG. 4. Appalachian *Vittaria* sporophyte from sterile culture. ×5.2. FIGS. 5 and 6. Enlarged view of clathrate scales. ×260.

Spontaneous callus formation was observed in the cultures kept by one of us (Whitten) in three out of nine tubes maintained for seven months on media containing 2% sucrose without transfer to fresh media. The callus (*Fig. 2*) formed a friable mass varying in color from yellow to dark brown, and appeared to originate from the center of the near-spherical mass of gametophytes. Adjacent to the callus tissue toward the center of the prothallial mass, occasional aberrant gametophytes with enlarged, irregular cells occurred. A total of six sporophytes were produced in two of the three callus-containing cultures after about nine months. The sporophytes arose from the edge or center of a mass of callus tissue, and did not appear to be associated with normal gametophyte tissue. The largest sporophyte had produced fronds up to 7 mm in length when all cultures died during an air conditioning failure in the laboratory.

Before death, the young sporophytes (*Figs. 3* and *4*) had produced a distinct rhizome which bore clathrate scales (*Figs. 5* and *6*), which are typical of the genus *Vittaria*. No roots were observed. The fronds were linear or unequally dichotomously branched, and each contained a distinct vascular bundle.

Since the sporophytes appeared to arise from callus tissue and no sex organs were observed, it is presumed that they arose apogamously. Further work on living material is needed to determine their exact origin and ploidy. The production of presumably apogamous sporophytes on Appalachian *Vittaria* gametophytes has been noted in both natural populations (Farrar, 1978) and in material cultured under non-sterile conditions by Alma S. Stokey, as cited by Farrar (1978). In both instances, the sporophytes aborted after producing a few small fronds. The sporophytes in our cultures were also small, but appeared healthy until their untimely death. In all instances, the sporophytes were too immature to provide taxonomic data useful at the species level. Attempts to obtain viable, mature sporophytes are in progress. The appearance of sporophytes in our old, crowded, and tightly closed cultures suggests that ethylene may play a role in callus formation and apogamy. Ethylene is produced by a wide range of plants and causes many different responses (Burg, 1962). Moreover, Elmor and Whittier (1973) have demonstrated that ethylene can induce apogamy in gametophytes of *Pteridium aquilinum* (L.) Kuhn. They further demonstrated this effect in nine of its strains (Elmore & Whittier, 1975). Future experiments involving ethylene are needed not only on Appalachian *Vittaria* gametophytes but also on other gametophytes of the southern Appalachians in the genera *Hymenophyllum*, *Trichomones*, and *Grammitis* (Farrar, 1967).

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REVIEW

"THE FERN GENUS DAVALLIA IN CULTIVATION [DAVALLIACEAE]," "DAVALLIA RELATIVES IN CULTIVATION: ARAIOSTEGIA, DAVALLODES, HUMATA, AND SCYPHULARIA [DAVALLIACEAE]," and **"THE GENUS PYRROSIA IN CULTIVATION [POLYPODIACEAE],"** by Barbara Joe Hoshizaki, Baileya 21:1–42, 43–50, and 53–76. 1981.—These papers continue the author's long series of useful treatments of cultivated ferns. Each paper contains an introduction, key to the species, and a brief synonymy and notes on the morphology, distribution, and cultivation of each species and its cultivars. The cultivars, although not included in the key, are distinguished in the text and, like the wild species, are illustrated with silhouettes. Anyone needing to identify cultivated Davalliaceae and *Pyrrosia* will find these papers invaluable.—*D.B.L.*